REPETITIVE TESTICULAR BIOPSY IN THE RAM DURING PUBERTAL DEVELOPMENT

D. D. Lunstra and S. E. Echternkamp

U.S. Department of Agriculture, Agricultural Research Service
Roman L. Hruska U.S. Meat Animal Research Center,
Clay Center, NE 68933

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ABSTRACT

A procedure for testicular biopsy was developed and tested in rams at 14, 18 and 22 wk of age. The biopsy procedure produced a tissue sample with minimal cell-to-cell disruption and caused no detectable detriment to testicular development in rams. At least three biopsies from the same testis were obtained at 4-wk intervals without influencing the developmental patterns of either the biopsied or nonbiopsied testicle. Data obtained by biopsy indicated that the testes of Suffolk rams reached a comparable stage of development (22 wk of age) approximately 4 wk later than the testes of Finnsheep rams (18 wk of age). These results demonstrate that repetitive testicular biopsy can be performed successfully in the ram during pubertal development. The biopsy procedure allows repeated sampling of the same animal and offers a reasonable alternative to castration.

Key words: biopsy, testis, repeat, histology, ram

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INTRODUCTION

Testicular biopsy in the bull, boar and other domestic animals has been relatively unsuccessful (1-8) because the resulting hematoma, inflammation, and lesions cause serious damage to the testis (1-4, 8, 9) and the tissue sample obtained is grossly disrupted (3, 4, 6, 7). Successful repetitive testicular biopsy has been reported in the rabbit (9, 10), and testicular biopsy in man is widely used and has considerable diagnostic value (4, 11, 12). The success of testicular biopsy in the rabbit and in man is due to the presence of a less complex pattern of blood vessels in the vascular layer of the tunica albuginea (9, 11), making the selection of a relatively avascular area for an incision in these species much easier than it would be in the testis of domestic animals.

The purpose of this study was to develop an effective technique for testicular biopsy, using the ram as a model, and to evaluate the effects of repeated biopsy on the ram testis during pubertal development.

MATERIALS AND METHODS

Twenty-five Suffolk and 10 Finnsheep (Finn) ram lambs born in May were assigned to the experiment. Rams were weaned at approximately 8 wk of age and maintained on a standard growing ration through the end of the experiment. Twenty-four rams (10 Finn, 14 Suffolk) were assigned to receive a single testicular biopsy at 18 wk of age, followed immediately by castration. Samples of tissue were obtained at castration for comparison to the tissue obtained by biopsy from the same testis. Eleven additional Suffolk rams were assigned to receive repetitive unilateral testicular biopsy at 14, 18 and 22 wk of age. Immediately after biopsy at 22 wk, the 11 rams were castrated and samples of tissue from both testes were fixed for comparison.

Testicular biopsy was performed unilaterally on rams under general anesthesia (Halothane). Rams were placed in a supine position on a surgical table, the scrotum was shaved and scrubbed, and the scrotum and testes were drawn through an opening in a surgical drape to lie on a sterile surgical field. A 2-cm incision was made through the scrotum over the testis at its widest diameter. An avascular area of the tunica vaginalis was located and a 1-cm opening was made with scissors. Small Allis forceps were used to grasp the lips of the incision in the tunica vaginalis. The testis was manipulated by palpation of the cauda epididymis through the scrotum until a relatively avascular area (3 to 5 mm diameter) of the tunica albuginea was located. Avascular areas of the tunica albuginea were generally found in the medial portion of the midsagittal suface of the testis, since the tunica albuginea tended to thicken near the areas where the epididymis attached to the testicular capsule. Blunt dissection of the selected area was performed by pressing the closed tips of a small iris scissors perpendicularly against the tunica albuginea and then opening the tips approximately 3 mm. This process was repeated several times,

rotating the plane of the tips approximately 90 degrees each time, until a 3-mm diameter opening in the tunica albuginea was produced without hemorrhage and without disrupting the underlying parenchyma. The edge of the opening was grasped with small mouse-toothed iris forceps, and a 10-G biopsy needle[a] was inserted slowly but firmly to a depth of 1 cm while performing 90 degree reciprocating rotations. The biopsy needle then was rotated approximately three revolutions and the needle containing the tissue sample was withdrawn. The cylindrical mass of testicular tissue (approximately 3mm x 10mm) was immediately expelled from the biopsy needle with a stylet and processed for histology.

A small amount of antibiotic suspension (0.5 ml)[b] was placed directly on the biopsy site and spread between the testicular capsule and the tunica vaginalis by moving the latter with Allis forceps. Additional antibiotic suspension (0.5 ml) was spread between the scrotal lining and the tunica vaginalis, and only the outer periphery of the incision in the scrotum was sutured.

Testicular tissue obtained by biopsy and samples (2 x 2 x 4 mm) of tissue obtained at castration were fixed with 2% glutaraldehyde in 75 mM cacodylate buffer (pH 7.4), post-fixed with 1% osmium tetroxide, dehydrated through a graded ethanol series, and embedded in Araldite-502 resin mixture (13). Sections (2 µm thick) were taken at approximately 300-µm intervals and stained with toluidine blue; then photomicrographs were prepared at 200x magnification. Diameters of 50 to 100 seminiferous tubules cut at right angles to their long axes in each embedded sample were measured, and the percentage of tubules containing spermatids with condensed, elongated nuclei was assessed to obtain the proportion of tubules exhibiting complete spermatogenesis. Data were analyzed using least squares procedures (14), and subgroup means were compared using Student's t-test (15).

RESULTS

Characteristics of testicular tissue obtained by biopsy did not differ ($P>0.20$) from those of tissue obtained by castration immediately after biopsy (Table 1). At 18 wk of age, Finn rams exhibited more advanced testicular development than Suffolk rams, and biopsy tissue within breed provided histological data that did not differ from that of castration tissue within breed.

Tissue obtained by biopsy exhibited minimal disruption of cell-to-cell relationships and did not differ in histological appearance from that of tissue obtained by castration (Figure 1).

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[a]Custom fabricated needle, 10 G, thinwall, 90 degree blunt tip, four-inch length (Popper & Sons, Inc., New Hyde Park, NY). The blunt tip was hollow-ground and sharpened using a conical grindstone to produce a 30-degree internal bevel.

[b]Forte-Topical antibiotic, oil suspension (The UpJohn Company, Kalamazoo, MI).

Table 1. Characteristics of testicular tissue obtained by biopsy and by castration of Finn and Suffolk rams at 18 wk of age[a]

Characteristic	No. of rams	Biopsy tissue[b]	Castration tissue
Average tubule diameter (μm)			
Finn rams	10	199 ± 10	208 ± 10
Suffolk rams	14	172 ± 8	173 ± 8
All rams	24	188 ± 8	191 ± 8
Tubules with ES (%)			
Finn rams	10	36.6 ± 9.1 (7)	37.5 ± 9.1 (7)
Suffolk rams	14	13.9 ± 7.3 (4)	14.5 ± 7.3 (4)
All rams	24	25.0 ± 6.8 (11)	25.7 ± 6.8 (11)

[a]Least squares means ± SEM. Both the biopsy and castration tissues were obtained from the same testis. ES = elongated spermatids.
[b]Number of rams exhibiting tubules with ES is given in parentheses.
[b]Not different (P>0.20) from castration tissue.

Table 2. Characteristics of the repetitively biopsied testis at 14, 18 and 22 wk and of the nonbiopsied contralateral testis at 22 wk of age in Suffolk rams[a]

Characteristic	Biopsied testis 14 wk	Biopsied testis 18 wk	Biopsied testis 22 wk	Nonbiopsied testis (22 wk)[b]
Testis weight (g)	nc	nc	151 ± 20	158 ± 20
Epididymal weight (g)	nc	nc	21 ± 2	22 ± 2
Testis length (cm)	6.8 ± 0.3[c]	7.7 ± 0.4[c]	8.4 ± 0.5[c]	8.4 ± 0.5
Testis diameter (cm)	3.9 ± 0.2[c]	5.4 ± 0.2[c]	6.3 ± 0.3[c]	6.4 ± 0.3
Tubule diameter (μm)	125 ± 10	170 ± 11	215 ± 7	232 ± 7
Tubules with ES (%)[d]	0 ± 0(0)	13 ± 8(3)	58 ± 11(8)	74 ± 11(8)

[a]Least squares means ± SEM (n = 11 rams). All rams were subjected to unilateral biopsy of the left testis at 14, 18 and 22 wk of age.
[b]nc = data not collected. ES = elongated spermatids.
[c]Obtained at castration. Not different (P>0.20) from 22-wk biopsy.
[c]Obtained by caliper measurement at surgery.
[d]Number of rams exhibiting tubules with ES is given in parentheses.

Disruption of structure was noted at the periphery of specimens obtained by biopsy and by castration, and evaluation of tissue within approximately 300 μm of the specimen periphery was avoided. The cylindrical tissue mass (weight = approximately 70 mg) obtained by biopsy was limited to approximately 3 mm diameter by 10 mm length, and the total number of seminiferous tubules present per cross section decreased from approximately 100 tubules at 14 wk to 20 to 30 tubules at 22 wk of age in Suffolk rams (Figure 1). However, the amount and integrity of the testicular tissue obtained by biopsy was adequate for histological evaluation in all age groups.

Repetitive biopsy did not impair testicular development, since no differences ($P>0.20$) existed between the biopsied and the nonbiopsied testis at 22 wk of age (Table 2) in the 11 Suffolk rams subjected to three successive testicular biopsies. Testicular size, diameter of seminiferous tubules and percentage of tubules containing elongated spermatids were not affected by the biopsy procedure employed at 14, 18 and 22 wk of age. At castration (22 wk), one ram exhibited adhesion between the tunica vaginalis and the surface site

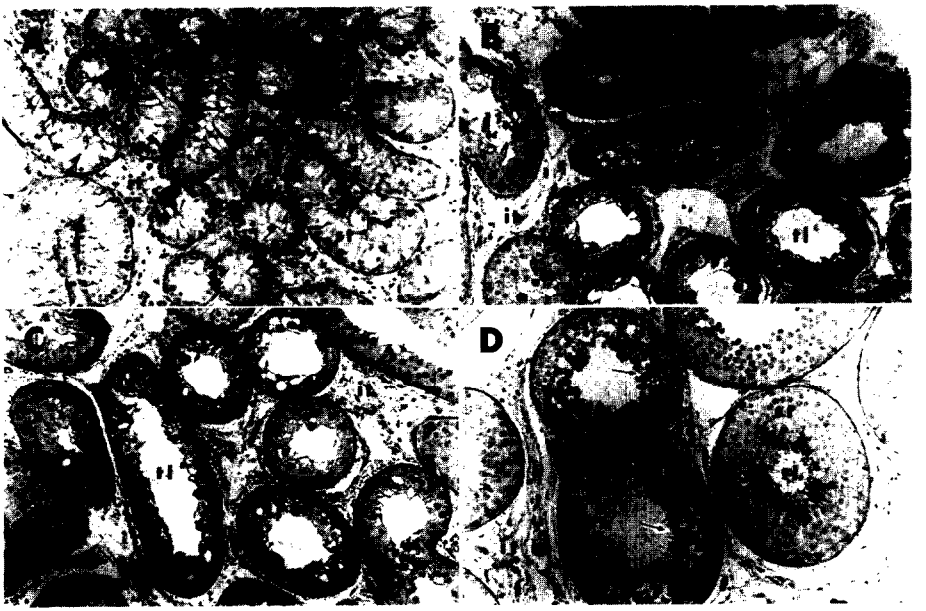

Figure 1. Photomicrographs of testicular tissue from Suffolk rams during pubertal development. Magnification = 100x. A) Ram #139 biopsy tissue, 14 wk of age; B) Ram #168 biopsy tissue, 18 wk of age; C) Ram #168 castration tissue, 18 wk of age; D) Ram #139 biopsy tissue, 22 wk of age. Note that elongated spermatids did not appear within majority of seminiferous tubules until 22 wk age. Symbols: Seminiferous tubule (st), elongated spermatids (arrows), tubule lumen (tl), interstitial tissue (it).

of the biopsy taken at 18 wk of age, but mobility and development of the testis was not affected. The other biopsy sites (14 and 18 wk) in all rams had healed without adhesions and a smooth covering of tunica albuginea had been reestablished over each of the sites. Histological examination of testicular tissue underlying the surface of the 14-wk biopsy sites revealed a barely discernable streak of connective tissue along the path of the biopsy, with no evidence of granuloma or residual inflammation due to the biopsy.

Linear increases ($P<0.01$) in testicular dimensions, diameter of seminiferous tubules and percentage of tubules containing elongated spermatids occurred between 14 and 22 wk of age (Table 2). Comparison of data (Tables 1 and 2) indicates that Suffolk rams (22 wk) achieved comparable pubertal development approximately 4 wk later than Finn rams (18 wk of age).

DISCUSSION

Hemorrhage at biopsy and postoperative inflammation, adhesions, and lesions are the general causes of the testicular damage that have occurred with previously reported biopsy techniques in domestic animals (4, 9). The success of our technique in the ram is attributed to the avoidance of hemorrhage by using blunt dissection during biopsy to minimize damage to the vascular layer of the tunica albuginea, the reduction of postoperative inflammation and adhesions by using an antibiotic-oil suspension to lubricate and promote aseptic healing, and the reduction of foreign-body irritation by using sutures only at the scrotal surface.

The tissue samples obtained with this biopsy technique displayed little disruption, were adequate for histological evaluation, and represented the functional status of the ram testis well enough to equal that observed in tissue obtained by castration. Samples of testicular tissue obtained by this biopsy technique should also be adequate for ultrastructural evaluation, microdetermination of steroids in testicular tissue, and in vitro incubations (4). The absence of testicular damage with repetitive biopsy should make this the procedure of choice where repetitive sampling is desirable or when castration must be avoided.

Onset of puberty has been reported to be earlier in Finn rams than in other breeds of sheep (16, 17), and the testicular characteristics obtained in this experiment are similar to those previously reported for Suffolk (18) and Finn (16, 17) rams during pubertal development. The similarity between the testicular characteristics of Finn rams at 18 wk (Table 1) and Suffolk rams at 22 wk of age (Table 2) indicate that Finn rams reach puberty approximately 4 wk earlier than Suffolk rams. This procedure for testicular biopsy should prove beneficial to future studies of testis function in the ram and may have application in other domestic species.

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